

In the Claims:

Please amend claims ~~3~~, ~~5~~, ~~7~~ to ~~14~~, ~~17~~, ~~18~~, ~~20~~, ~~21~~, ~~23~~, ~~25~~ to ~~28~~, ~~32~~, ~~34~~, and ~~36~~ as shown on the attached "Claim Sheets Marked up to Show Changes".

Please add new claims ~~39~~ to ~~42~~ as shown in the attached Clean Claim Sheets which show all claims pending in the application, after entry of this amendment.

REMARKS

This preliminary amendment is being submitted concurrently with Applicants' Response to the Notice to File Missing Parts. Applicants submit that this preliminary amendment is appropriate under 37 C.F.R. § 1.115 and request that it be entered.

This application is the United States national phase application corresponding to PCT/EP99/00945.

Claims 3, 5, 7 to 14, 17, 18, 20, 21, 23, 25 to 28, 32, 34 and 36 have been amended to eliminate certain multiple dependencies and references to multiple dependencies in multiple dependent claims and to put the claims in better form for examination under United States practice. Applicants submit that these amendments are supported by the specification and give rise to no issue of new matter.

New claims 39 to 42 have been added to more particularly point out and distinctively claim certain aspects of Applicants' invention. Applicants submit that these claims are clearly supported by the specification and give rise to no issue of new matter.

If the Examiner believes that a telephone interview would expedite prosecution of this application, he is encouraged to telephone the undersigned Applicants' attorney.

If the fees submitted are incorrect or if any other fees are due in connection with this submission, please charge any fee or credit any overpayment to Deposit Account No. 12-2475.

Respectfully submitted,

LYON & LYON LLP

Dated: January 29, 2001

By: 

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Serial No. 09/622,101

Claim Sheets Marked up to Show Changes

3. (Amended) The method of claim 1 [or 2], wherein said nucleotide is a nucleoside monophosphate.
5. (Amended) The method of [any one of claims 1 to 4] claim 3, wherein said protein is derived from a eukaryotic or prokaryotic organism.
7. (Amended) The method of [any one of claims 1 to 6] claim 5, wherein said protein comprises the amino acid sequence of any one of SEQ ID NOS: 1 to 13 or a fragment thereof.
8. (Amended) The method of [any one of claims 1 to 7] claim 5, wherein said amino acid which is substituted or added in (a) is glutamic acid, glycine or lysine and the amino acid substituted in (c) is tyrosine.
9. (Amended) The method of [any one of claims 1 to] claim 8, wherein said amino acid which is substituted or added in (b) is a basic amino acid, preferably arginine.
10. (Amended) The method of [any one of claims 1 to] claim 9, where the LID has the consensus sequence R/KXXXXXXERYEXXXQ.
11. (Amended) The method of [any one of claims 1 to] claim 10, wherein said polypeptide exhibits kinase activity for a nucleoside or nucleotide analog which is higher than that of [the/]a corresponding wild type eukaryotic[, preferably human] enzyme.

12. (Amended) The method of [any one of claims 1 to 11] claim 10, wherein said nucleoside analog is AZT, d4T or has the following structure:



wherein B is any nucleobase or analog thereof, and X is O, CH<sub>2</sub>, NH or S.

13. (Amended) The method of [any one of claims 1 to] claim 12, wherein the amino acid substitution(s) result in the P-loop and/or in the LID region of a bacterial nucleoside or nucleotide kinase[, preferably those of the TmpK of E.coli].
14. (Amended) A polynucleotide encoding the polypeptide obtainable by the method of [any one of claims 1 to 13] claim 10.
17. (Amended) The vector of claim [15 or] 16, which is a gene transfer or a gene targeting vector.
18. (Amended) A host cell genetically engineered with the vector of [any one of claims 15 to 17] claim 16.
20. (Amended) A method for producing cells capable of expressing a polypeptide having nucleoside or nucleotide kinase activity for nucleoside or nucleotide analogs comprising genetically engineering cells with [the

polynucleotide of claim 14, or with] the vector of [any one of claims 15 to 17] claim 16.

21. (Amended) A polypeptide having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog encoded by a polynucleotide of claim 14[, obtainable by the method of any one of claims 1 to 13 or 19 or from cells produced by the method of claim 20] or [comprising] a biologically active fragment [of any of these] thereof.
23. (Amended) A composition comprising
- (a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide; or
  - (b) the polypeptide of claim 21[, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17];
  - (c) optionally a nucleoside or nucleotide analog; and
  - (d) optionally a pharmaceutically acceptable carrier.
25. (Amended) The composition of claim [23 or] 24, wherein said protein is a bacterial TmpK.
26. (Amended) The composition of [any one of claims 23 to] claim 25, wherein said protein has at least the P-loop and/or the LID region of E.coli TmpK.
27. (Amended) The composition of claim 25 [or 26], wherein said TmpK comprises the amino acid sequence shown in SEQ ID NO: 4 or a biologically active fragment thereof.

28. (Amended) A kit comprising [the polynucleotide of claim 14,] the vector of [any one of claims 15 to 17, the protein of claim 21 or the antibody of claim 22,] claim 16 and optionally a nucleoside or nucleotide analog.

32. (Amended) Use of

(a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide, or

(b) the polypeptide of claim 21[, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17; and/or

(c) the nucleoside or nucleotide analog identified in the method of claim 30]

for the preparation of a pharmaceutical composition for the activation of nucleoside or nucleotide analogs or nucleoside or nucleotide based prodrugs [and/or for the treatment of viral infections and/or diseases or cancer].

34. (Amended) The use of claim [32 or] 33, wherein said viral infection is HIV infection.

36. (Amended) A method for the preparative synthesis of a nucleoside phosphate analog comprising:

(a) using a polynucleotide of claim 14 [or as defined in any one of claim 23 to 27] in a noncellular system or in a cell *ex vivo*, and

(b) formulating the cells modified in step (a) in a pharmaceutically acceptable form.

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Clean Claim Sheets

1. A method for the production of a polypeptide having or having enhanced kinase activity for a nucleoside or nucleotide analog, said method comprising substituting, adding or deleting at least one amino acid of a protein having nucleoside or nucleotide kinase activity at a position in the protein where:
- (a) the amino acid is at position  $X_2$  and/or  $X_3$  in the consensus sequence  $GX_1X_2X_3X_4GK$  of the P-loop;
  - (b) the amino acid is in the LID region; and/or
  - (c) the amino acid is at position 105 in the amino acid sequence of human thymidylate kinase or at a corresponding position in a protein having nucleoside or nucleotide kinase activity.

2. The method of claim 1, wherein said nucleoside is adenosine, cytidine, guanosine, thymidine or uridine or based on any of these.

- A1 3. (Amended) The method of claim 1, wherein said nucleotide is a nucleoside monophosphate.

4. The method of claim 3, wherein said nucleoside monophosphate is thymidylate.

- A2 5. (Amended) The method of claim 3, wherein said protein is derived from a eukaryotic or prokaryotic organism.

6. The method of claim 5, wherein said organism is human or a yeast.

7. (Amended) The method of claim 5, wherein said protein comprises the amino acid sequence of any one of SEQ ID NOS: 1 to 13 or a fragment thereof.

8. (Amended) The method of claim 5, wherein said amino acid which is substituted or added in (a) is glutamic acid, glycine or lysine and the amino acid substituted in (c) is tyrosine.

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9. (Amended) The method of claim 8, wherein said amino acid which is substituted or added in (b) is a basic amino acid, preferably arginine.

10. (Amended) The method of claim 9, where the LID has the consensus sequence R/KXXXXXERYEXXXQ.

11. (Amended) The method of claim 10, wherein said polypeptide exhibits kinase activity for a nucleoside or nucleotide analog which is higher than that of a corresponding wild type eukaryotic enzyme.

12. (Amended) The method of claim 10, wherein said nucleoside analog is AZT, d4T or has the following structure:





wherein B is any nucleobase or analog thereof, and X is O, CH<sub>2</sub>, NH or S.

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Amended

13. (Amended) The method of claim 12, wherein the amino acid substitution(s) result in the P-loop and/or in the LID region of a bacterial nucleoside or nucleotide kinase.

14. (Amended) A polynucleotide encoding the polypeptide obtainable by the method of claim 10.

15. A vector containing the polynucleotide of claim 14.

16. The vector of claim 15, wherein the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells.

17. (Amended) The vector of claim 16, which is a gene transfer or a gene targeting vector.

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18. (Amended) A host cell genetically engineered with the vector of claim 16.

19. A method for producing a polypeptide having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog comprising

- (a) culturing the host cell of claim 18, and
- (b) recovering said polypeptide from the culture.

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20. (Amended) A method for producing cells capable of expressing a polypeptide having nucleoside or nucleotide kinase activity for nucleoside or nucleotide analogs



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27. (Amended) The composition of claim 25, wherein said TmpK comprises the amino acid sequence shown in SEQ ID NO: 4 or a biologically active fragment thereof.

28. (Amended) A kit comprising the vector of claim 16 and optionally a nucleoside or nucleotide analog.

29. A method for identifying an inhibitor of a nucleoside or nucleotide kinase comprising the steps of:

- (a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a compound to be screened under conditions that permit binding of said compound to the nucleoside or nucleotide kinase, and
- (b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the absence or decrease of the signal is indicative for an inhibitor of a nucleoside or nucleotide kinase.

30. A method for identifying a nucleoside or nucleotide based prodrug comprising the steps of

- (a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a nucleoside or nucleotide analog to be screened under conditions that permit kinase activity of said polypeptide, and
- (b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the presence of a signal is indicative for a putative prodrug.

31. A method for the production of a pharmaceutical composition comprising the steps of

- (a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a compound to be screened under conditions that permit binding of said compound to the nucleoside or nucleotide kinase, and
- (b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the absence or decrease of the signal is indicative for an inhibitor of a nucleoside or nucleotide kinase, or
- (a') contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a nucleoside or nucleotide analog to be screened under conditions that permit kinase activity of said polypeptide, and
- (b') detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the presence of a signal is indicative for a putative prodrug; and
- (c) formulating the inhibitor identified in step (b) or the nucleoside or nucleotide analog identified in step (b') in a pharmaceutically acceptable form.

32. (Amended) Use of

- (a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing

AS  
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said protein *in vivo* or a vector containing said polynucleotide, or

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(b) the polypeptide of claim 21  
for the preparation of a pharmaceutical composition for the activation of nucleoside or nucleotide analogs or nucleoside or nucleotide based prodrugs.

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33. The use of claim 32, wherein said activation results in a cytotoxic nucleoside or nucleotide.

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34. (Amended) The use of claim 33, wherein said viral infection is HIV infection.

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35. Use of the inhibitor obtainable by the method of claim 31 for the preparation of a pharmaceutical composition for inhibiting virus replication or for treating cancer.

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36. (Amended) A method for the preparative synthesis of a nucleoside phosphate analog comprising:

- AIW
- (a) using a polynucleotide of claim 14 in a noncellular system or in a cell *ex vivo*, and
  - (b) formulating the cells modified in step (a) in a pharmaceutically acceptable form.
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37. The composition of any one of claims 23 to 27 or the use of any one of claims 32 to 34, wherein said composition is a pharmaceutical composition and further comprises or is designed to be administered with a nucleoside or nucleotide analog, preferably AZT or d4T.

38. Use of

- (a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide,
- (b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17 for the preparation of nucleoside phosphates or analogs and derivatives thereof.

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39. The method of claim 10 wherein said protein comprises the amino acid sequence of any one of SEQ. ID. NOS. 1 to 13 or a fragment thereof.

40. The method of claim 11 wherein said enzyme is a human enzyme.

41. The method of claim 13 wherein said bacterial kinase is a Tmpk of E.coli.

42. The use according to claim 32 wherein said pharmaceutical composition is used for the treatment of a viral infection or disease for the treatment of cancer.

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